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MOELLMAN SPRAY CHAMBER: versatile research tool for laboratory bioassays

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Laboratory spray chambers have been useful tools in insecticide research.¹ Until 1973, we used three kinds of spray chambers in our work. The dimensions, design, and construction of these units—the Potter Spray Tower² and two glass spray chambers³—restricted the type of spray operation for which they could be used. With the Potter Spray Tower, for example, only objects held in a 100-mm diameter petri dish could be sprayed. Furthermore, the walls of this unit could not be cleaned without taking apart the chamber—an operation that could expose the operator to toxic substances. The glass spray chambers had similar disadvantages, complicated by their use of a nebulizer for atomization of sprays. The nebulizer worked well for atomizing liquids, such as water or light spray oils, but worked poorly for viscous liquids, such as mineral oil.

In 1973, we designed, built, and began using the Moellman Spray Chamber,⁴ which incorporated as many qualities as we could identify as necessary for a laboratory spray unit. The general design followed that of Nigam,⁵ but was highly modified to our specifications. Our most important considerations were accuracy, safety, and utility.

The Moellman Spray Chamber has proved itself to be a useful tool for laboratory bioassays of insecticides. The qualities which we found outstanding in more than 5 years of use are its stainless steel construction, automatic application system, and safety. We have used the Chamber in insecticide bioassays with insects, branchlets of foliage, and small potted seedlings.

Other investigators will undoubtedly find other applications for the chamber. Adaptations of our design or operation procedures may be necessary to meet particular needs. For example, spraying suspensions of microbials or viruses may require more thorough cleaning of the spray chamber than we have found necessary in application of insecticides.

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The Moellman Spray Chamber can apply precise amounts of insecticides to insects and small trees in the laboratory. Variables in its operation were defined in calibration tests. The results showed that significant differences in the amount of insecticide deposited on the specimen table resulted from the volatility of the solvent sprayed and not from the method of deposit assessment used or from the substrate sprayed. The height of the specimen table significantly affected spray volume deposited, but rotation of the specimen table during spray application did not. Since 1973, when the Moellman Spray Chamber was developed, it has proved its usefulness in laboratory bioassays. Other investigators will undoubtedly find other applications for the chamber.

Retrieval Terms: bioassay; laboratories; insecticides; spray chambers; Moellman Spray Chamber.

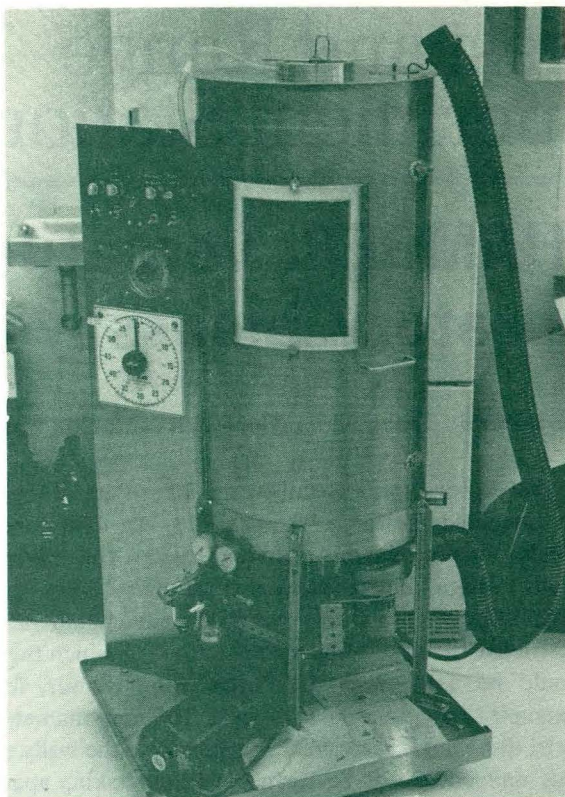


Figure 1— Moellman Spray Chamber.

DESIGN AND OPERATION

A primary feature of the Moellman Spray Chamber (figs. 1 and 2) is a 25.4-cm diameter specimen table which can accommodate a variety of objects such as potted trees, dishes of insects, glass plates, or other surfaces for deposit assessment. The specimen table can be adjusted to vary the distance between the specimen and the spray nozzle. Spray deposited on the plane of the specimen table is relatively uniform, as judged by dye deposits.

Toxic aerosols and vapors are removed automatically from the chamber after each spray application. The delay between spray application and evacuation of spray vapors can be varied from 1 second to 60 minutes.

The stainless steel construction and configuration of the spray chamber permit easy cleaning and decontamination of all inner surfaces. After spray application, clean solvent is sprayed on the inner surfaces with a squirt bottle. The solvent washes the surfaces and flows out of the chamber through the trap into a waste collection receptacle (fig. 2).

We have adapted two atomization systems to this spray chamber. Both use a commercially available fine Potter spray nozzle for atomization. The first system, which we use for volumes greater than 250

μl , is the conventional Potter arrangement with nozzle, siphon tube, and reservoir.² The second system (fig. 3), fitted with a 10 to 500 μl syringe, can be used to deliver 10 to 500 μl into the nozzle. The vacuum generated by the air stream passing through the nozzle evacuates the solvent from the syringe when the operator moves the plunger to the full position. As the solvent begins moving, the operator releases the plunger, and it moves inward to the empty position. Liquid in the syringe is then atomized through the nozzle.

A typical sequence of steps in operating the spray chamber when the target is to be exposed to the spray for 60 seconds before evacuation of the vapors is as follows (fig. 2):

First: The spray chamber is plugged into a 100-volt AC outlet, the desired volume added to the reservoir (21 b) or syringe, and the turntable plate, or specimen table (7), is adjusted to the desired height.

Second: The object to be sprayed is placed on the specimen table and the door (5) securely closed.

Third: The main power switch (15) is turned on.

Fourth: The exhaust timer (18) is set to 50 seconds and the spray timer (19) to 10 seconds.

Fifth: This arrangement results in a 10-second atomization time with a 50-second delay before activation of the exhaust pump.

Sixth: The exhaust timer, air pump, and turntable (optional) switches on panel 17 are turned on.

Seventh: The spray timer switch (19) is turned to "On," and the air pump remains on for 10 seconds at 10 psi.

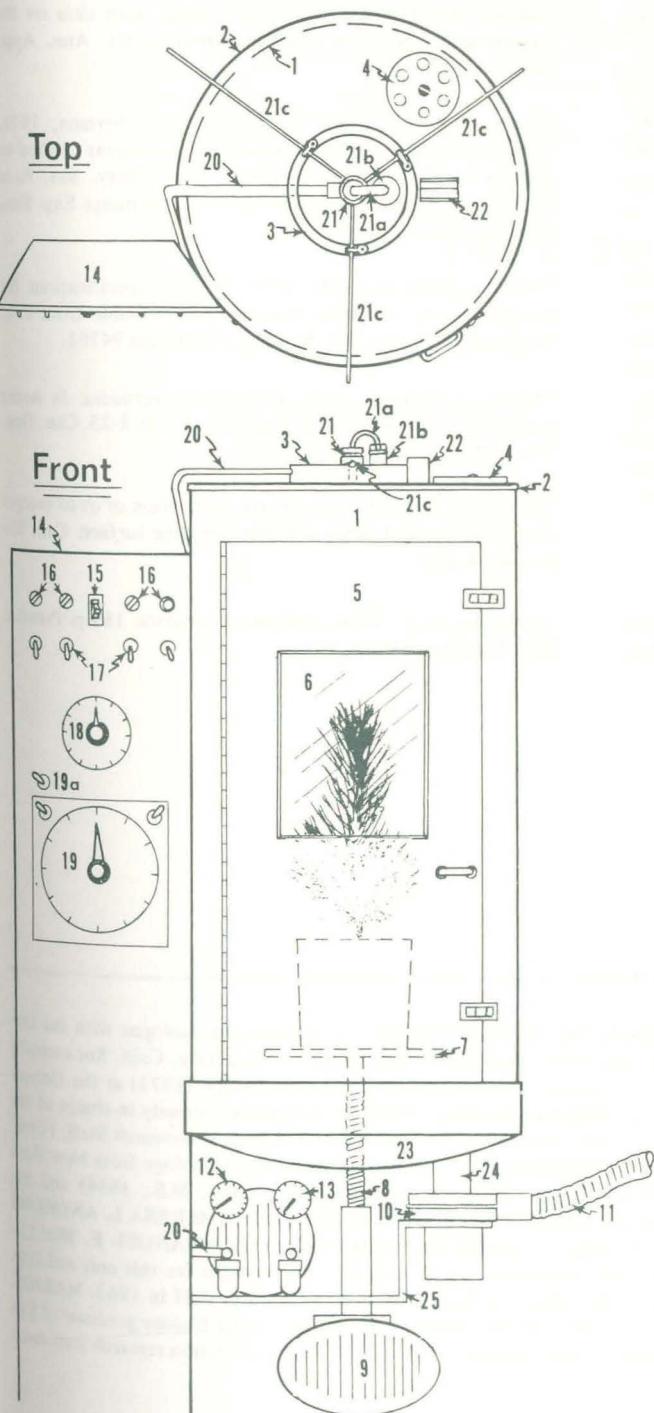
Eighth: The operator then turns the spray timer switch to "Off."

Ninth: The exhaust pump will come on automatically 50 seconds after the air pump shuts off, and will remain on until the air pump is activated in the next spray cycle.

Tenth: Air pressure within the chamber is equalized with ambient air pressure by opening the vent (4). The necessity for an elaborate manometer system has thus been eliminated.

CALIBRATION OF THE SPRAY CHAMBER

Several parameters of the spray chamber's operation were assessed by a series of calibration experiments. We were concerned with the relative accuracy of three methods of deposit assessment. Also, we wanted to determine the effects of delivery volume, turntable height, and turntable speed on the spray volume deposited.



Legend

1. Chamber—91.41 by 45.72 cm i.d. stainless steel sheet 0.16 cm thick.
2. Lid—48.26 cm d.
3. Ring on lid—15.24 cm d., 1.27 cm thick, 2.22 cm high, holding Potter spray nozzle.
4. Vent on lid—10.16 cm d., six 1.27-cm d. holes on 3.18-cm r.
5. Door of chamber—76.20 cm high by 50.80 cm on arc (40.64-cm cord). Door held with stainless steel piano hinge 3.81 cm.
6. Glass in door—opening 25.40 cm high, 20.32 cm wide. Glass is curved, 30.48 cm high by 25 cm wide, and sealed to door with teflon gasket.
7. Turntable plate—25.40 cm d.
8. Turntable stem—2.54 cm d.
9. Turntable motor—90° drive unit, 0 to 60 r/min.
10. Exhaust blower—12.70 by 5.08 cm (Flexible tube exhausts fumes to atmosphere or fume-hood.)
11. Flexible tube—5.08 cm.
12. "Gast" air pump (supplies 0 to 30 psi.).
13. "Gast" vacuum (supplies 0 to 76.20 cm mercury) pump.
14. Instrument panel—All electrical components are explosion proof to permit spraying flammable fluids.
15. Main power switch.
16. Four fuses.
17. Four control switches.
18. Exhaust timer.
19. Spray timer with control switches.
- 19a. Spray timer on/off switch.
20. Feeder hose—air—tygon tubing to spray nozzle.
21. Potter spray nozzle—Siphon tube (21a) connects nozzle to reservoir (21b); nozzle is supported by rods (21c).
22. Resting block for microliter syringe.
23. Floor of chamber—dished for cleaning and draining excess fluids to outside of container.
24. Drain trap.
25. Mounting bracket.

Figure 2— Schematic of the Moellman Spray Chamber.

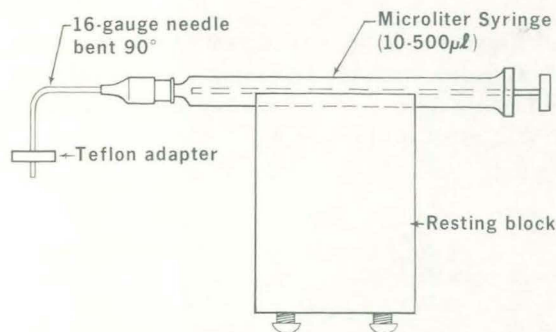


Figure 3—Schematic of the Microvolume Fluid Application System.

Comparison of Analytical Methods

To determine the relative accuracy of three methods of deposit assessment—weight,² dye,⁶ and gas-liquid chromatography (GLC)—we performed an experiment in which the experimental model was one with the three factors crossed. For each method of deposit assessment, the effects of solvent volatility and deposit substrate were compared. We assumed that GLC would provide the most accurate assessment because it is chemically precise. Results from the other methods would be compared to GLC and their relative accuracies determined.

Three solvents of different volatilities were tested: TPM (tripropylene glycol monomethyl ether) (specific gravity, 0.965), TPM:kerosene (1:1) (S.G., 0.877), and TPM:kerosene (1:9) (S.G., 0.816). Oil red dye was mixed in each solvent at a concentration of 5 mg/ml, and the carbamate insecticide aminocarb at a concentration of 1 mg/ml. A constant volume of 250

µl was sprayed using the microliter syringe delivery system. Sixteen applications using each of the three solvents were made with glass and filter paper targets, four applications on each of 4 separate days.

Spray assessments with two deposit substrates were done simultaneously. Filter paper discs 9 cm in diameter were sprayed together with 8.89-cm square glass plates. Weight of spray deposited was determined to the nearest 0.1 mg immediately after application, and spray weight converted directly to µg aminocarb per unit area. The aminocarb and dye on both substrates were then eluted with 5 ml of analytical grade chloroform. An aliquot of the aminocarb-dye-chloroform solution was then subjected to colorimetric analysis to determine µg aminocarb per unit area using the procedure of Raynor.⁶ The aliquot was then added back into the larger sample.

Next, samples were analyzed by GLC by evaporating the chloroform a volume of 1 ml, and injecting 1 µl into a Varian Aerograph gas chromatograph. Temperatures were (°C): column, 175; detector, 200; and injector, 190. Gas flows were (ml/min): N, 40; air, 50; H, 300. A 5 percent OV 101 on gas chrom Q column was used, and amounts of aminocarb were then calculated by the Spectra-Physics Autolab system IVB chromatography data analyzer. Values found were then corrected to reflect the area of the substrate surface.

The calculated weights of aminocarb per unit area were compared for solvent, analytical method, and substrate with an analysis of variance and Scheffe's test of means.⁷ Solvent was the only significant factor affecting spray deposit (table 1). Spray deposited with TPM:kerosene (1:9) was significantly less than

Table 1—Calculated weight of aminocarb deposited on two substrates by the Moellman Spray Chamber using three solvents¹ (N=16 applications for each entry)

Method of analysis ²	TPM sprayed on . . . ²		TPM:K (1:) sprayed on . . . ²		TPM:K (1:9) sprayed on . . . ²	
	Glass	Paper	Glass	Paper	Glass	Paper
	µg/cm ² (mean±S.E.)					
Weight	0.54±0.01	0.51±0.01	0.56±0.01	0.54±0.02	0.51±0.02	0.46±0.02
Dye	0.63±0.02	0.60±0.34	0.44±0.06	0.30±0.05	0.25±0.08	0.14±0.03
GLC	0.57±0.01	0.52±0.01	0.45±0.04	0.41±0.03	0.29±0.03	0.25±0.02

¹Analysis of Variance

	F	d.f.
Solvent	94.06 ^a	2,6
Method	4.25	2,6
Substrate	3.65	1,3

^aSignificant at the 0.1 percent level.

²GLC = gas liquid chromatography; TPM = tripropylene glycol monomethyl ether; K = kerosene

with TPM:kerosene (1:1). The deposit with TPM:kerosene (1:1) was significantly less than TPM alone (Scheffe's test of means). These differences may have resulted from greater vaporization of the more volatile solvents during atomization. The drop spectra of the more volatile solvents would, therefore, consist of smaller droplets which would remain suspended in the spray chamber longer than the larger droplets of less volatile solvents. As a result, the settling times of more volatile solvents would be longer, and less toxicant would contact the deposit substrate per unit time. This explanation, rather than evaporation after deposition, is supported by the distinct progression of average deposits determined by dye and GLC for the three progressively less volatile solvents. The lack of a distinct progression in values determined by weight of spray cannot be explained.

Other Variables of Operation

We determined the relationship of delivery volume to volume deposited on a 9-cm diameter filter paper disc by spraying 0.125, 0.250, and 0.500 ml of TPM containing 10 mg/ml oil red dye. Weight and dye assessments were made as described above, except that dye was eluted with a 10-ml reagent grade toluene. Five assessments with each volume and each analytical method were done on three separate occasions. Results were consistent with our previous findings (table 2). Volumes sprayed significantly affected volumes deposited, but assessment methods did not differ from one another.

Effects of turntable height were assessed by spraying 0.50 ml of TPM containing 5 mg/ml oil red dye on a 9-cm diameter filter paper disc. Ten assessments

at the lowest, middle, and highest levels were made by colormetric analysis. The volumes deposited were significantly different from one another ($F = 64.592$; d.f. = 2,27; $p < 0.01$):

Level of turntable:	Volume deposited (Mean±S.E.) (gal/acre)
lowest	1.69±0.08
middle	2.10±0.10
highest	3.34±0.13

Finally, turntable speed effects were determined with 10 deposits each at 0 and 1/5 revolutions per minute (rpm). A volume of 0.05 ml containing 5 mg/ml oil red dye was sprayed on 9-cm diameter filter paper discs. The volumes deposited did not differ significantly from one another ($F = 2.092$; d.f. = 1,18; $p > 0.01$):

Turntable speed:	Volume deposited (Means±S.E.) (gal/acre)
stationary	1.60±0.06
1/5 rpm	1.52±0.04

DISCUSSION

We defined certain basic variables of operating the spray chamber, but others, such as droplet spectra of various solvents, have yet to be explored. To date, we know that solvent volatility, volume sprayed, and height of the specimen table significantly affect spray deposit; substrate surface used for assessment, method of determining spray deposit, and rotation of the turntable plate do not. Our discovery that spray deposits varied relative to height within the spray chamber complicated experiments with objects that have height. Spray deposit at the top of an object will be higher than at the bottom. In spraying potted trees, for example, the top of the tree is likely to receive more spray than the lower branches. Since we cannot account for variations in branch configurations and obstructions to spray delivery at lower planes of the tree, and for variations in total surface area, we used a conservative approach for estimating deposit on trees. We assessed deposit at the base of the pot, that is, on the plane of the turntable platform. This procedure gave us an estimate of the minimum probable deposit on the tree. Other users of the Moellman Chamber may prefer to assess deposit

Table 2—Effect of volume sprayed on volume deposited on a 9-cm diameter filter paper disc in the Moellman Spray Chamber¹ (N=15 applications)

Method of analysis	Volume sprayed (ml)		
	0.125	0.250	0.500
	Volume deposited (gal/acre) (mean±S.E.)		
Weight	0.80±0.09	1.34±0.09	2.42±0.19
Dye	0.76±0.05	1.55±0.18	2.49±0.11

¹Analysis of variance

	F	d.f.
Volume sprayed	159.39 ^a	2,3
Method	0.579	1,72

^aSignificant at the 1 percent level.

at a different plane. So long as a single plane is used in all experiments, continuity and comparability among and within experiments will be maintained.

An investigator using the Moellman Chamber can choose any of three deposit assessment methods—weight, dye, or GLC—with equal accuracy. We used each of these methods successfully, and make no recommendations as to which of them should be adopted. Two concerns, however, have led us to use the dye method routinely. Weight determinations may change because of evaporation if not done immediately after deposition on the assessment surface. This consideration particularly pertains to the use of extremely evaporative solvents such as acetone. GLC, the most sophisticated of the methods, is no more accurate than the dye method, but is substantially more expensive and time-consuming.

NOTES

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⁴ Patent applied for June 1975. Detailed specifications for construction of the spray chamber are available from E. E. Moellman, P.O. Box 245, Berkeley, California 94701.

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⁶ Raynor, A. C. 1956. *Colorimetric estimation of dyed insecticide spray deposit using a paper sampling surface*. Can. Entomol. 88:279.

⁷ Guenther, W. C. 1964. *Analysis of variance*. 199 p. Prentice Hall, Englewood Cliffs, N.J.

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